

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Divisional Application of:

Application No.: 09/449,437

Filed: November 24, 1999

For: NOVEL ANTIBODIES AND LIGANDS FOR "BONZO"  
CHEMOKINE RECEPTOR

Date: August 27, 2001

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PRELIMINARY AMENDMENT

Box PATENT APPLICATION

Assistant Commissioner for Patents

Washington, D.C. 20231

Sir:

Please amend the subject application as follows:

In the Specification

Please add the following paragraph at page 1, after the title:

RELATED APPLICATIONS

This application is a divisional of U.S. Application No. 09/449,437, filed November 24, 1999, the entire teachings of which are incorporated herein by reference.

Please replace the paragraph at page 4, lines 6 through 16 with the following paragraph:

The invention also relates to an isolated cell that produces an antibody or antigen-binding fragment of the present invention, including those which bind to mammalian Bonzo and inhibit the binding of a ligand to the receptor. In one embodiment, the isolated cell is murine hybridoma 4A11 (also referred to as murine hybridoma LS212-4A11-30-8) deposited under ATCC Accession No. PTA-991. In another embodiment, the isolated cell is murine hybridoma 7A2 (also referred to as murine hybridoma LS212-7A2-32-1) deposited under ATCC Accession No. PTA-992. In another embodiment, the isolated cell is murine hybridoma 7F3 (also referred to as murine hybridoma LS212-7F3-8-7) deposited under ATCC Accession No. PTA-990. In another embodiment, the isolated cell is murine hybridoma 9G2 (also referred to as murine hybridoma LS212-9G2-7-2).

Please replace the paragraph at page 5, lines 9 through 18 with the following paragraph:

The invention also relates to therapeutic methods in which agents which can bind to a mammalian Bonzo and modulate (inhibit or promote) a Bonzo function are administered to a subject in need of such therapy. In one embodiment, the therapeutic method is a method of treating a subject having an inflammatory disease. In another embodiment, the subject has a cancer or an infection (e.g., viral, bacterial, fungal). In another embodiment, the therapeutic method is a method of inhibiting a cellular response (e.g.,  $\text{Ca}^{2+}$  flux, chemotaxis, exocytosis, respiratory burst). In another embodiment, the method is a method of modulating a Bonzo

function. In another embodiment, SExCkine is locally administered to a subject to recruit Bonzo<sup>+</sup> cells to the area of administration.

Please replace the paragraph at page 6, lines 1 through 4 with the following paragraph:

The invention also relates to therapeutic methods in which targeting molecules are administered to a subject in need of such therapy. In one embodiment, the therapeutic method is a method of treating a subject having a tumor or a viral infection.

Please replace the paragraph at page 12, lines 3 through 9 with the following paragraph:

Figures 26A-26F are fluorescence histograms showing that Bonzo expression is augmented by repeated activation of *in vitro* derived TR1 cells. Cells which had been stimulated by one round of activation (Figures 26A-26C) or two rounds of activation (Figures 26D-26F) were stained with anti-Bonzo mAb 7F3 (Figures 26B and 26E), anti-CCR4 mAb 1G1 (Figures 26A and 26D) or anti-CCR7 mAb 7H12 (Figures 26C and 26F). TR1 cells expressed increased amounts of Bonzo after repeated activation (compare Figures 26B and 26E).

Please replace the paragraph at page 19, lines 23 through 28 with the following paragraph:

"Receptor-binding variants" of mammalian SExCkine proteins include receptor-binding fragments (e.g., proteolytic fragments), receptor-binding mutant proteins and receptor-binding fusion proteins which can be produced using suitable methods (e.g., mutagenesis (e.g., chemical mutagenesis, radiation mutagenesis), recombinant DNA techniques). A "receptor-binding variant" can be identified using a suitable receptor-binding assay such as a Bonzo-binding assay described herein.

Please replace the paragraph at page 32, lines 10 through 16 with the following paragraph:

mAb 4A11 can be produced by murine hybridoma 4A11, also referred to as murine hybridoma LS212-4A11-30-8, which was deposited on November 24, 1999, on behalf of LeukoSite, Inc., 215 First Street, Cambridge, MA 02142, U.S.A. (now Millennium Pharmaceuticals, Inc., 75 Sidney Street, Cambridge, MA 02139, U.S.A.), at the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110, U.S.A., under Accession No. PTA-991. The invention relates to murine hybridoma 4A11, to the antibody it produces and to nucleic acids encoding the antibody.

Please replace the paragraph at page 32, lines 17 through 22 with the following paragraph:

mAb 7A2 can be produced by murine hybridoma 7A2, also referred to as murine hybridoma LS212-7A2-32-1, which was deposited on November 24, 1999, on behalf of LeukoSite, Inc., 215 First Street, Cambridge, MA 02142, U.S.A. (now Millennium Pharmaceuticals, Inc., 75 Sidney Street, Cambridge, MA 02139, U.S.A.), at the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110, U.S.A., under Accession No. PTA-992. The invention relates to murine hybridoma 7A2, to the antibody it produces, and to nucleic acids encoding the antibody.

Please replace the paragraph at page 32, lines 23 through 28 with the following paragraph:

mAb 7F3 can be produced by murine hybridoma 7F3, also referred to as murine hybridoma LS212-7F3-8-7, which was deposited on November 24, 1999, on behalf of LeukoSite, Inc., 215 First Street, Cambridge, MA 02142, U.S.A. (now Millennium Pharmaceuticals, Inc., 75 Sidney Street, Cambridge, MA 02139, U.S.A.), at the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110, U.S.A., under Accession No. PTA-990. The invention relates to murine hybridoma 7F3, to the antibody it produces, and to nucleic acids encoding the antibody.

Please replace the paragraph at page 33, line 28 through page 34, line 14 with the following paragraph:

The invention also relates to a bispecific antibody, or functional fragment thereof (e.g.,  $F(ab')_2$ ), which binds to a mammalian Bonzo and at least one other antigen (e.g., tumor antigen, viral antigen). In a particular embodiment, the bispecific antibody, or functional fragment thereof has the same or similar epitopic specificity as mAb 4A11, mAb 7A2, mAb 7F3 or mAb 9G2 and at least one other antibody. Bispecific antibodies can be secreted by triomas and hybrid hybridomas. Generally, triomas are formed by fusion of a hybridoma and a lymphocyte (e.g., antibody secreting B cell) and hybrid hybridomas are formed by fusion of two hybridomas. Each of the fused cells (i.e., hybridomas, lymphocytes) produces a monospecific antibody. However, triomas and hybrid hybridomas can produce an antibody containing antigen binding sites which recognize different antigens. The supernatants of triomas and hybrid hybridomas can be assayed for bispecific antibody using a suitable assay (e.g., ELISA), and bispecific antibodies can be purified using conventional methods. (see, e.g., U.S. Patent No. 5,959,084 (Ring *et al.*) U.S. Patent No. 5,141,736 (Iwasa *et al.*), U.S. Patent Nos. 4,444,878, 5,292,668, 5,523,210 (all to Paulus *et al.*) and U.S. Patent No. 5,496,549 (Yamazaki *et al.*)).

Please replace the paragraph at page 34, line 15 through page 35, line 8 with the following paragraph:

In another embodiment, the antibody or antigen-binding fragment thereof has specificity for a mammalian SExCkine, preferably a naturally occurring or endogenous human SExCkine. Such antibodies and antigen-binding fragments can be produced by a variety of suitable methods, such as those described herein. In one embodiment, the anti-SExCkine antibody can be raised against an appropriate immunogen, such as an isolated soluble and/or recombinant SExCkine or portions thereof (including synthetic molecules, such as synthetic peptides). Antibodies can also be raised by immunizing a suitable animal (e.g., mouse) with cells which express the transmembrane form of SExCkine. In another embodiment, the antibody is an IgG or antigen-binding fragment of an IgG. In another embodiment, the antibody is a human antibody or an

antigen-binding fragment thereof. In another embodiment, the antibody is a humanized antibody or an antigen-binding fragment thereof. In a preferred embodiment, the antibody or antigen-binding fragment can bind to a mammalian SExCkine and inhibit (reduce or prevent) the binding of the chemokine to receptor (e.g., Bonzo), and thereby inhibit one or more functions mediated by receptor in response to SExCkine binding. For example, the anti-SExCkine antibody can inhibit SExCkine-induced chemotaxis of Bonzo<sup>+</sup> cells. Other functions which can be mediated by SExCkine binding to receptor (e.g., Bonzo) include, for example, signal transduction (e.g., GDP/GTP exchange by receptor associated G proteins, transient increase in the concentration of cytosolic free calcium [Ca<sup>2+</sup>]<sub>i</sub>) and receptor-mediated processes and cellular responses (e.g., proliferation, migration, chemotaxis, secretion, degranulation, inflammatory mediator release (such as release of bioactive lipids such as leukotrienes (e.g., leukotriene C<sub>4</sub>)), respiratory burst).

Please replace the paragraph at page 38, lines 9 through 17 with the following paragraph:

The first binding moiety can be, for example, an antibody which binds mammalian Bonzo or antigen-binding fragment thereof (e.g., Fab, Fv, Fab', F(ab')<sub>2</sub>), a Bonzo ligand (e.g., mammalian SExCkine, mammalian platelet factor 4) or Bonzo-binding variant of a ligand. The second binding moiety can be, for example, an antibody or antigen-binding fragment thereof which binds to a molecule expressed on the target cell or antigen binding fragment thereof. Where the targeting molecule comprises a first binding moiety which is an anti-Bonzo antibody or antigen-binding fragment thereof, it is preferred that said anti-Bonzo antibody does not inhibit binding of ligand to Bonzo.

Please replace the paragraph at page 54, lines 15 through 17 with the following paragraph:

In another embodiment, the invention relates to a method of promoting Bonzo mediated homing of leukocytes in a subject, comprising administering an effective amount of a promoter (e.g., agonist) of Bonzo function.

Please replace the paragraph at page 57, lines 14 through 17 with the following paragraph:

- immunosuppression, such as that in individuals with immunodeficiency syndromes such as AIDS, individuals undergoing radiation therapy, chemotherapy, or other therapy which causes immunosuppression; immunosuppression due to congenital deficiency in receptor function or other causes.

Please replace the paragraph at page 66, lines 17 through 27 with the following paragraph:

Human multiple tissue northern blots I and II and a cancer cell line blot (Clontech) were used to analyze expression of the gene encoding the Bonzo ligand. cDNA probes were labeled with  $\alpha^{32}\text{P}$ -dCTP by priming with random hexamers. A 400 bp fragment representing most of the chemokine domain of SExCkine cDNA cloned in pCDEF3 (from the 5' EcoR1 site (within vector pCDEF3) to an EcoRV site of a cDNA encoding human SExCkine (SEQ ID NO: 3)) was used as the hybridization probe for all blots. Hybridization was performed at 68°C for 1 hour in ExpressHyb (Clontech) with denatured probe at a concentration of  $1 \times 10^6$  CPM/mL. Blots were then washed for 20 minutes in 2 X SSC/0.05% SDS at room temperature followed by high stringency washes at 50°C, 60°C, or 65°C in 0.1 X SSC/0.1% SDS for 20 minutes per wash and exposed to Kodak XAR film with an intensifying screen.

Please replace the paragraph at page 67, lines 3 through 7 with the following paragraph:

Fusion proteins consisting of amino terminal regions of SExCkine fused to a C-terminal Histadine (His) were made in pEF-His or pEF1/V5-His A from Invitrogen (Carlsbad, CA) and fusion proteins consisting of N-terminal SExCkine regions fused to human alkaline phosphatase with a C-terminal His tag were made in the pDERF-SEAP vector (Yoshie, O., *et al.*, *J. Leukoc. Biol.*, 62(5):634-644 (1997)).

Please replace the paragraph at page 67, lines 8 through 16 with the following paragraph:

The alkaline phosphatase fusion was produced by amplifying human SExCkine cDNA (SEQ ID NO:3) by PCR using a 5' synthetic oligonucleotide primer that contained a Sall site (5'cgcgctcgacagccgagatgggacgggacttg3', SEQ ID NO:12) and a 3' synthetic oligonucleotide primer that contained a XbaI site (5'ggtctagatgtcctggctgtgggacca3', SEQ ID NO:13). The 5' primer (SEQ ID NO:12) annealed to nucleotides 15-29 of SEQ ID NO:3 and encodes a protein beginning at the initiating Met (amino acid residue 1 of SEQ ID NO:4) and the 3' primer (SEQ ID NO:13) annealed to nucleotides 602-622 of SEQ ID NO:3. The PCR was run for 30 cycles (95°C (30 seconds), 55°C (30 seconds), 72°C (1 minute)).

Please replace the paragraph at page 67, line 25 through page 68, line 7 with the following paragraph:

Additional constructs encoding fragments of the extracellular domain of SExCkine were made by PCR using a 5' primer (SEQ ID NO:14) and synthetic primer KHLL 3' (5' ggt cta gaa agt aaa tgc ttc tgg tgg gc 3', SEQ ID NO:16) or synthetic primer LMS 3' (5' cct cta gag ctc atc aat tcc tga acc c 3', SEQ ID NO:17) or synthetic primer 155 3' (5' ggt cta gac tgg gag ggt ggg gcg ctg ag 3', SEQ ID NO:18). Primer KHLL 3' annealed to nucleotides 345-364 of SEQ ID NO:3, and the product of the amplification reaction encoded residues 1 to 117 of SExCkine (SEQ ID NO:4). Primer LMS 3' annealed to nucleotides 280-300 of SEQ ID NO:3, and the product of the amplification reaction encoded residues 1 to 95 of SExCkine (SEQ ID NO:4). Primer 155 3' annealed to nucleotides 457-477 of SEQ ID NO:3, and the product of the amplification reaction encoded residues 1 to 155 of SExCkine (SEQ ID NO:4). Primers KHLL 3', LMS 3' and 155 3' each contained an XbaI restriction site.

Please replace the paragraph at page 68, line 19 through page 69, line 4 with the following paragraph:



Thirty 10 cm plates (Beckton Dickinson) were seeded with  $1 \times 10^6$  293T cells in DMEM +10% FCS. The next day the 293T cells were transfected by adding 10  $\mu$ g SExCkine/SEAP DNA to 790  $\mu$ L opti-MEM (800  $\mu$ L total) and mixing it with a solution of 60  $\mu$ L LipofectAMINE™ 2000 in 740  $\mu$ L opti-MEM (800  $\mu$ L total). The mixture was incubated at room temperature for 30 min, an additional 6.4 mL of opti-MEM was added to the mixture, and the mixture was added to the plates containing 293T cells. The plates were incubated at 37°C for 3 hours, then 8.0 mL DMEM+ 20% FCS was added. 24 hours later the transfection mixture was removed, the plates were washed with 1 X PBS, and 10 mL of serum free DMEM were added. The plates were then incubated for 3 days. The media (culture supernatant) was removed and filtered (500 mL filter bottle) to remove cellular debris. The harvested media was assayed for chemotactic activity using Bonzo/L1.2 cells essentially as described above. In addition, dilution curves were generated using supernatant diluted in media in a range of undiluted to 1:16 to assess general activity.

Please replace the paragraph at page 70, line 19 through page 71, line 6 with the following paragraph:

A panel of antibodies which bind human Bonzo were produced by immunizing mice with transfected L1.2 cells that expressed high levels of Bonzo. The antibodies specifically bound to Bonzo expressed on the surface of Bonzo/L1.2 cells (Figures 8A-8D) but did not bind to transfected L1.2 cells which expressed CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, GPR5, V28, GPR9-6, Bob, LyGPR, AF, APJ or RDC (Figures 9A-9G). The antibodies, (e.g., mAbs 4A11, 7A2 and 7F3) inhibited the binding of SExCkine to Bonzo (Figure 10, Figure 31, Table 1). Staining studies revealed that Bonzo is expressed on small populations of CD4<sup>+</sup> and CD8<sup>+</sup> T cells as well as on CD16<sup>+</sup>/CD56<sup>+</sup> NK cells. However, no expression of Bonzo was observed on CD19<sup>+</sup>/CD20<sup>+</sup> B cells or on CD14<sup>+</sup> monocytes (Figures 11A-11H). Multi-color staining studies were performed to analyze the co-expression of Bonzo and other cell surface proteins (Figures 12A-12D, 13A-13J, 14A-14H, 15A-15C, 16A-16D). These studies revealed that Bonzo is expressed predominantly on CD45RO<sup>hi</sup> memory lymphocytes. Furthermore, Bonzo expression was detected on both skin

homing (CLA<sup>+</sup>) and gut homing ( $\alpha 4\beta 7^{+}$  and  $\alpha E^{+}$ ) CD4<sup>+</sup> lymphocytes (Figures 12A-12D). Bonzo was co-expressed with CCR1, CCR2, CCR5, CCR6, CXCR1, CXCR2 or CXCR3 on lymphocytes (Figures 13A-13J).

Please replace the paragraph at page 72, lines 9 through 17 with the following paragraph:

This study demonstrates that recombinant SExCkine and recombinant proteins encoding parts of the amino terminal portion of SExCkine can be used in conjunction with Bonzo (e.g., a cell expressing Bonzo) in receptor binding assays and functional assays to screen for potential agonists and antagonists of Bonzo. Considering that Bonzo is highly expressed on all classes of chronically stimulated T cell subsets, antagonists of the receptor (e.g., mAbs 4A11, 7A2 and 7F3) can be administered to treat chronic inflammatory diseases. Furthermore, agonists of Bonzo (e.g., SExCkine) can be administered to recruit killer T cell subsets to, for example, solid tumors or sites of infection.

Amendments to the specification are indicated in the attached "Marked Up Version of Amendments" (pages i-ix).

#### In the Claims

Please cancel Claims 1-15, 17-20, 48-50, 52, 54-57, 59, 61-66, 68, 70-76, 78, 79, 81-83, 85-87, 95 and 96. Claims 24, 27-33, 35, 43-46, 84 and 88 have been amended and are presented below in amended form and Claims 97-112 have been added. In accordance with 37 C.F.R. § 1.121(c)(1)(ii), amendments to the claims are indicated in the attached "Marked Up Version of Amendments" (pages x-xii).

24. (Amended) The antibody or antigen-binding fragment of Claim 21 wherein said antibody or antigen-binding fragment inhibits a cellular response to binding of ligand to said Bonzo in an *in vitro* assay with an IC<sub>50</sub> of less than about 7 µg/mL.

27. (Amended) The antibody or antigen-binding fragment of Claim 21 wherein the binding of said antibody or said antigen-binding fragment to Bonzo can be inhibited by an antibody selected from the group consisting of mAb 4A11, the antibody produced by the hybridoma cell line deposited as ATCC Accession Number PTA-991, mAb 7A2, the antibody produced by the hybridoma cell line deposited as ATCC Accession Number PTA-992 and mAb 7F3, the antibody produced by the hybridoma cell line deposited as ATCC Accession Number PTA-990.
28. (Amended) An antibody produced by murine hybridoma 4A11, deposited as ATCC Accession Number PTA-991, or an antigen-binding fragment thereof.
29. (Amended) An antibody produced by murine hybridoma 7A2, deposited as ATCC Accession Number PTA-992, or an antigen-binding fragment thereof.
30. (Amended) An antibody produced by murine hybridoma 7F3, deposited as ATCC Accession Number PTA-990, or an antigen-binding fragment thereof.
31. (Amended) Murine hybridoma 4A11, deposited as ATCC Accession Number PTA-991.
32. (Amended) Murine hybridoma 7A2, deposited as ATCC Accession Number PTA-992.
33. (Amended) Murine hybridoma 7F3, deposited as ATCC Accession Number PTA-990.
35. (Amended) The isolated cell of Claim 34 wherein said antibody or antigen-binding fragment inhibits a cellular response to binding of ligand to said Bonzo *in vitro* assay with an IC<sub>50</sub> of less than about 7 µg/mL.
43. (Amended) The antibody or antigen-binding fragment of Claim 42 wherein said antibody or antigen-binding fragment thereof inhibits a cellular response in an *in vitro* assay with an IC<sub>50</sub> of less than about 7 µg/mL.

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44. (Amended) The antibody or antigen-binding fragment of Claim 42 wherein said cellular response is chemotaxis.
45. (Amended) The antibody or antigen-binding fragment of Claim 41 wherein said mammalian Bonzo is human Bonzo.
46. (Amended) The antibody or antigen-binding fragment of Claim 41 wherein said ligand is SExCkine.
84. (Amended) A test kit for use in detecting the presence of mammalian Bonzo or portion thereof in a biological sample comprising
- a) an antibody or antigen-binding fragment thereof which binds to mammalian Bonzo and inhibits binding of a ligand to said mammalian Bonzo; and
  - b) one or more ancillary reagents suitable for detecting the presence of a complex between said antibody or antigen-binding fragment and said mammalian Bonzo.
88. (Amended) The test kit of Claim 84 wherein said antibody or antigen-binding fragment is selected from the group consisting of
- a) mAb 4A11, the antibody produced by the hybridoma cell line deposited as ATCC Accession Number PTA-991;
  - b) mAb 7A2, the antibody produced by the hybridoma cell line deposited as ATCC Accession Number PTA-992;
  - c) mAb 7F3, the antibody produced by the hybridoma cell line deposited as ATCC Accession Number PTA-990;
  - d) an antibody which can compete with mAb 4A11, mAb 7A2 or mAb 7F3 for binding to mammalian Bonzo; and
  - e) an antigen-binding fragment of (a), (b), (c) or (d).

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97. (New) The antibody or antigen-binding fragment of Claim 21 wherein said antibody or antigen-binding fragment inhibits a cellular response to binding of ligand to said Bonzo in an *in vitro* assay with an  $IC_{50}$  of less than about 5  $\mu\text{g/mL}$ .
98. (New) The antibody or antigen-binding fragment of Claim 21 wherein said antibody or antigen-binding fragment inhibits a cellular response to binding of ligand to said Bonzo in an *in vitro* assay with an  $IC_{50}$  of less than about 1  $\mu\text{g/mL}$ .
99. (New) The isolated cell of Claim 34 which produces an antibody or antigen-binding fragment thereof, wherein said antibody or antigen-binding fragment inhibits a cellular response to binding of ligand to said Bonzo in an *in vitro* assay with an  $IC_{50}$  of less than about 5  $\mu\text{g/mL}$ .
100. (New) The isolated cell of Claim 34 which produces an antibody or antigen-binding fragment thereof, wherein said antibody or antigen-binding fragment inhibits a cellular response to binding of ligand to said Bonzo in an *in vitro* assay with an  $IC_{50}$  of less than about 1  $\mu\text{g/mL}$ .
101. (New) The antibody or antigen-binding fragment of Claim 41 wherein said antibody or antigen-binding fragment inhibits a cellular response to binding of ligand to said Bonzo in an *in vitro* assay with an  $IC_{50}$  of less than about 5  $\mu\text{g/mL}$ .
102. (New) The antibody or antigen-binding fragment of Claim 41 wherein said antibody or antigen-binding fragment inhibits a cellular response to binding of ligand to said Bonzo in an *in vitro* assay with an  $IC_{50}$  of less than about 1  $\mu\text{g/mL}$ .

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103. (New) The antibody or antigen-binding fragment of Claim 21 wherein said antibody or antigen-binding fragment has the epitopic specificity of an antibody selected from the group consisting of mAb 4A11, the antibody produced by the hybridoma cell line deposited as ATCC Accession Number PTA-991; mAb 7A2, the antibody produced by the hybridoma cell line deposited as ATCC Accession Number PTA-992; and mAb 7F3, the antibody produced by the hybridoma cell line deposited as ATCC Accession Number PTA-990.
104. (New) The antibody or antigen-binding fragment of Claim 103 wherein said antibody or antigen-binding fragment has the epitopic specificity of mAb 7F3, the antibody produced by the hybridoma cell line deposited as ATCC Accession Number PTA-990.
105. (New) The isolated cell of Claim 34 which produces an antibody or antigen-binding fragment thereof, wherein said antibody or antigen-binding fragment has the epitopic specificity of an antibody selected from the group consisting of mAb 4A11, the antibody produced by the hybridoma cell line deposited as ATCC Accession Number PTA-991; mAb 7A2, the antibody produced by the hybridoma cell line deposited as ATCC Accession Number PTA-992; and mAb 7F3, the antibody produced by the hybridoma cell line deposited as ATCC Accession Number PTA-990.
106. (New) The isolated cell of Claim 105 which produces an antibody or antigen-binding fragment thereof, wherein said antibody or antigen-binding fragment has the epitopic specificity of mAb 7F3, the antibody produced by the hybridoma cell line deposited as ATCC Accession Number PTA-990.

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107. (New) The antibody or antigen-binding fragment of Claim 41 wherein said antibody or antigen-binding fragment has the epitopic specificity of an antibody selected from the group consisting of mAb 4A11, the antibody produced by the hybridoma cell line deposited as ATCC Accession Number PTA-991; mAb 7A2, the antibody produced by the hybridoma cell line deposited as ATCC Accession Number PTA-992; and mAb 7F3, the antibody produced by the hybridoma cell line deposited as ATCC Accession Number PTA-990.
108. (New) The antibody or antigen-binding fragment of Claim 107 wherein said antibody or antigen-binding fragment has the epitopic specificity of mAb 7F3, the antibody produced by the hybridoma cell line deposited as ATCC Accession Number PTA
109. (New) The antibody or antigen-binding fragment of Claim 43 wherein said cellular response is chemotaxis.
110. (New) The antibody or antigen-binding fragment of Claim 21 wherein the binding of said antibody or said antigen-binding fragment to mammalian Bonzo can be inhibited by an antibody selected from the group consisting of mAb 4A11, the antibody produced by the hybridoma cell line deposited as ATCC Accession Number PTA-991; mAb 7A2, the antibody produced by the hybridoma cell line deposited as ATCC Accession Number PTA-992; and mAb 7F3, the antibody produced by the hybridoma cell line deposited as ATCC Accession Number PTA-990.
111. (New) The isolated cell of Claim 34 which produces an antibody or antigen-binding fragment thereof, wherein the binding of said antibody or said antigen-binding fragment to mammalian Bonzo can be inhibited by an antibody selected from the group consisting of mAb 4A11, the antibody produced by the hybridoma cell line deposited as ATCC Accession Number PTA-991; mAb 7A2, the antibody produced by the hybridoma cell line deposited as ATCC Accession Number PTA-992; and mAb 7F3, the antibody produced by the hybridoma cell line deposited as ATCC Accession Number PTA-990.

112. (New) The antibody or antigen-binding fragment of Claim 41 wherein the binding of said antibody or said antigen-binding fragment to mammalian Bonzo can be inhibited by an antibody selected from the group consisting of mAb 4A11, the antibody produced by the hybridoma cell line deposited as ATCC Accession Number PTA-991; mAb 7A2, the antibody produced by the hybridoma cell line deposited as ATCC Accession Number PTA-992; and mAb 7F3, the antibody produced by the hybridoma cell line deposited as ATCC Accession Number PTA-990.

#### REMARKS

The subject application is a divisional of U.S. Application No. 09/449,437, filed November 24, 1999. Original Claims 1-15, 17-20, 48-50, 52, 54-57, 59, 61-66, 68, 70-76, 78, 79, 81-83, 85-87, 95 and 96 have been cancelled. New Claims 97-112 have been added and are directed to the invention of Group III, drawn to an antibody or antigen-binding fragment thereof which binds Bonzo and inhibits ligand binding, kits comprising said antibody and a cell producing said antibody, as defined in the Restriction Requirement set forth in the Office Action dated March 15, 2001, which issued in parent U.S. Application No. 09/449,437.

#### Amendments to the Specification

The Specification has been amended to include a Related Applications paragraph, to recite ATCC Accession numbers and to correct certain informalities and typographical errors. Specifically:

At page 1, after the title, a RELATED APPLICATIONS paragraph claiming priority to U.S. Application No. 09/449,437, has been added.

At page 4, lines 9 and 10, the Specification has been amended to recite "murine hybridoma 4A11 (also referred to as murine hybridoma LS212-4A11-30-8) deposited under ATCC Accession No. PTA-991." Support for the recitation of ATCC Accession No. PTA-991 can be found in the copy of the ATCC Budapest Treaty Deposit Receipt and Viability Statement being filed concurrently herewith.

At page 4, lines 11 and 12, the Specification has been amended to recite "murine hybridoma 7A2 (also referred to as murine hybridoma LS212-7A2-32-1) deposited under ATCC

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Accession No. PTA-992.” Support for the recitation of ATCC Accession No. PTA-992 can be found in the copy of the ATCC Budapest Treaty Deposit Receipt and Viability Statement being filed concurrently herewith.

At page 4, lines 13 and 14, the Specification has been amended to recite “murine hybridoma 7F3 (also referred to as murine hybridoma LS212-7F3-8-7) deposited under ATCC Accession No. PTA-990.” Support for the recitation of ATCC Accession No. PTA-990 can be found in the copy of the ATCC Budapest Treaty Deposit Receipt and Viability Statement being filed concurrently herewith.

At page 5, line 13, “an” has been deleted and “a” has been inserted therefor.

At page 6, line 4, “an” has been deleted.

At page 12, lines 6 and 7, the Specification has been amended to recite “anti-CCR4 mAb 1G1.”

At page 19, line 25, “produce” has been deleted and “produced” has been inserted therefor.

At page 32, lines 11 and 12, lines 18-19 and lines 24-25, the Specification has been amended to recite “which was deposited on November 24, 1999, on behalf of LeukoSite, Inc., 215 First Street, Cambridge, MA 02142, U.S.A., at the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110, U.S.A. (now Millennium Pharmaceuticals, Inc., 75 Sidney Street, Cambridge, MA 02139, U.S.A.).”

At page 32, line 14, the Specification has been amended to recite “under Accession No. PTA-991.” Support for the recitation of ATCC Accession No. PTA-991 can be found in the copy of the ATCC Budapest Treaty Deposit Receipt and Viability Statement being filed concurrently herewith.

At page 32, line 21, the Specification has been amended to recite “under Accession No. PTA-992.” Support for the recitation of ATCC Accession No. PTA-992 can be found in the copy of the ATCC Budapest Treaty Deposit Receipt and Viability Statement being filed concurrently herewith.

At page 32, line 27, the Specification has been amended to recite “under Accession No. PTA-990.” Support for the recitation of ATCC Accession No. PTA-990 can be found in the

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copy of the ATCC Budapest Treaty Deposit Receipt and Viability Statement being filed concurrently herewith.

At page 34, line 4, “hybridomsa” has been deleted and “hybridomas” has been inserted therefor.

At page 34, line 27, “sexcikine” has been deleted and “SExCkine” has been inserted therefor.

At page 38, line 9, “and” has been deleted and “an” has been inserted therefor.

At page 54, line 15, the first occurrence of “a” has been deleted.

At page 57, lines 16 and 17, the Specification has been amended to recite “due to congenital deficiency”.

At page 66, line 18, “analyzed” has been deleted and “analyze” has been inserted therefor.

At page 67, line 6 and line 8, the Specification has been amended to recite “alkaline phosphatase.” Support for the recitation of “alkaline phosphatase” is found, for example, at page 69, lines 5-22.

At page 67, line 26 and page 68, line 2, line 4 and line 6, “SexCkine” has been deleted and “SExCkine” has been inserted therefor.

At page 68, line 25, “with” has been deleted. At page 68, line 27, “Plates” has been deleted and “plates” has been inserted therefor.

At page 70, line 29, “analyzed” has been deleted and “analyze” has been inserted therefor.

At page 72, line 11, “expression” has been deleted and “expressing” has been inserted therefor.

#### Amendments to the Claims

Original Claims 1-15, 17-20, 48-50, 52, 54-57, 59, 61-66, 68, 70-76, 78, 79, 81-83, 85-87, 95 and 96 have been cancelled. Claims 24, 27-33, 35, 43-46, 84 and 88 have been amended and Claims 97-112 have been added. Claims 16, 21-47, 51, 53, 58, 60, 67, 69, 77, 80, 84, 88-94 and 97-112 are pending.

Claims 24, 35 and 43 have been amended to recite “an IC<sub>50</sub> of less than about 7 µg/mL.” Support for the recitation can be found, for example, at page 31, lines 10-13.

Claims 27, 28, 31 and 88 have been amended to recite the ATCC Accession Number for murine hybridoma 4A11.

Claims 27, 29, 32 and 88 have been amended to recite the ATCC Accession Number for murine hybridoma 7A2.

Claims 27, 30, 33 and 88 have been amended to recite the ATCC Accession Number for murine hybridoma 7F3.

Claim 44 has been amended to depend from Claim 42.

Claims 45 and 46 have been amended to depend from Claim 41. Claim 46 has been further amended to include a period.

Claim 84 has been amended to delete “agent”, “or portion of said receptor” and “or a portion thereof, wherein said agent is not platelet factor-4.” Claim 84 has been further amended to recite “antibody or antigen-binding fragment thereof which binds to mammalian Bonzo and inhibits binding of a ligand to said mammalian Bonzo.” Support for the recitation can be found, for example, at page 30, line 21 to page 31, line 2 and in Claims 86 and 87 as originally filed.

Claim 88 has been amended to recite “antibody or antigen-binding fragment” and “an antigen-binding fragment of (a), (b), (c) or (d)” and to delete “which bind mammalian Bonzo or a portion thereof” and “combinations of the foregoing.” Claim 88 has been further amended to depend from Claim 84.

Support for new Claims 97-102 is found, for example, at page 31, lines 10-13.

Support for new Claims 103-108 is found, for example, at page 33, lines 14-27.

Support for new Claim 109 is found, for example, at page 23, line 20 *et seq.* and in Claim 44 as originally filed.

Support for new Claims 110-112 is found, for example, at page 31, lines 3-18.

The amendments to the specification and claims are supported by the parent application as originally filed. Therefore, this Amendment adds no new matter.

Information Disclosure Statement

An Information Disclosure Statement (IDS) is being filed concurrently herewith. Acknowledgment of consideration of the IDS is respectfully requested in the next Office Communication.

Biological Deposits

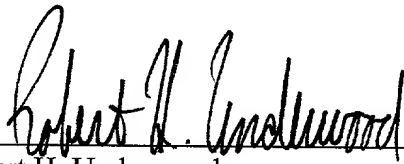
The application refers to murine hybridomas 7F3, 4A11 and 7A2 that were deposited at the American Type Culture Collection (ATCC) on November 24, 1999, under the provisions of the Budapest Treaty. A copy of the ATCC Budapest Treaty Deposit Receipt and Viability Statement for murine hybridomas 7F3, 4A11 and 7A2 is provided herewith. Further, a Statement Under 37 C.F.R. § 1.806 and 1.808 is being filed concurrently herewith, completing the formalities for deposit.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned at (781) 861-6240.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

By   
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Lexington, Massachusetts 02421-4799

Dated: *August 27, 2001*

MARKED UP VERSION AMENDMENTSSpecification Amendments Under 37 C.F.R. § 1.121(b)(1)(iii)

Please replace the paragraph at page 4, lines 6 through 16 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

The invention also relates to an isolated cell that produces an antibody or antigen-binding fragment of the present invention, including those which bind to mammalian Bonzo and inhibit the binding of a ligand to the receptor. In one embodiment, the isolated cell is murine hybridoma 4A11 (also referred to as murine hybridoma LS212-4A11-30-8) deposited under ATCC Accession No. [ ] PTA-991. In another embodiment, the isolated cell is murine hybridoma 7A2 (also referred to as murine hybridoma LS212-7A2-32-1) deposited under ATCC Accession No. [ ] PTA-992. In another embodiment, the isolated cell is murine hybridoma 7F3 (also referred to as murine hybridoma LS212-7F3-8-7) deposited under ATCC Accession No. [ ] PTA-990. In another embodiment, the isolated cell is murine hybridoma 9G2 (also referred to as murine hybridoma LS212-9G2-7-2).

Please replace the paragraph at page 5, lines 9 through 18 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

The invention also relates to therapeutic methods in which agents which can bind to a mammalian Bonzo and modulate (inhibit or promote) a Bonzo function are administered to a subject in need of such therapy. In one embodiment, the therapeutic method is a method of treating a subject having an inflammatory disease. In another embodiment, the subject has [an]a cancer or an infection (e.g., viral, bacterial, fungal). In another embodiment, the therapeutic method is a method of inhibiting a cellular response (e.g.,  $\text{Ca}^{2+}$  flux, chemotaxis, exocytosis, respiratory burst). In another embodiment, the method is a method of modulating a Bonzo function. In another embodiment, SExCkine is locally administered to a subject to recruit Bonzo<sup>+</sup> cells to the area of administration.

FOIA b 7 - D

Please replace the paragraph at page 6, lines 1 through 4 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

The invention also relates to therapeutic methods in which targeting molecules are administered to a subject in need of such therapy. In one embodiment, the therapeutic method is a method of treating a subject having [an] a tumor or a viral infection.

Please replace the paragraph at page 12, lines 3 through 9 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

Figures 26A-26F are fluorescence histograms showing that Bonzo expression is augmented by repeated activation of *in vitro* derived TR1 cells. Cells which had been stimulated by one round of activation (Figures 26A-26C) or two rounds of activation (Figures 26D-26F) were stained with anti-Bonzo mAb 7F3 (Figures 26B and 26E), anti-CCR4 mAb [mAb] 1G1 (Figures 26A and 26D) or anti-CCR7 mAb 7H12 (Figures 26C and 26F). TR1 cells expressed increased amounts of Bonzo after repeated activation (compare Figures 26B and 26E).

Please replace the paragraph at page 19, lines 23 through 28 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

"Receptor-binding variants" of mammalian SExCkine proteins include receptor-binding fragments (e.g., proteolytic fragments), receptor-binding mutant proteins and receptor-binding fusion proteins which can be [produce]produced using suitable methods (e.g., mutagenesis (e.g., chemical mutagenesis, radiation mutagenesis), recombinant DNA techniques). A "receptor-binding variant" can be identified using a suitable receptor-[receptor ]binding assay such as a Bonzo-binding assay described herein.

Please replace the paragraph at page 32, lines 10 through 16 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

mAb 4A11 can be produced by murine hybridoma 4A11, also referred to as murine hybridoma LS212-4A11-30-8, which was deposited on November 24, 1999, on behalf of LeukoSite, Inc., 215 First Street, Cambridge, MA 02142, U.S.A. (now Millennium Pharmaceuticals, Inc., 75 Sidney Street, Cambridge, MA 02139, U.S.A.), at the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110, U.S.A., under Accession No.[ ] PTA-991. The invention relates to murine hybridoma 4A11, to the antibody it produces and to nucleic acids encoding the antibody.

Please replace the paragraph at page 32, lines 17 through 22 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

mAb 7A2 can be produced by murine hybridoma 7A2, also referred to as murine hybridoma LS212-7A2-32-1, which was deposited on November 24, 1999, on behalf of LeukoSite, Inc., 215 First Street, Cambridge, MA 02142, U.S.A. (now Millennium Pharmaceuticals, Inc., 75 Sidney Street, Cambridge, MA 02139, U.S.A.), at the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110, U.S.A., under Accession No.[ ] PTA-992. The invention relates to murine hybridoma 7A2, to the antibody it produces, and to nucleic acids encoding the antibody.

Please replace the paragraph at page 32, lines 23 through 28 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

mAb 7F3 can be produced by murine hybridoma 7F3, also referred to as murine hybridoma LS212-7F3-8-7, which was deposited on November 24, 1999, on behalf of LeukoSite, Inc., 215 First Street, Cambridge, MA 02142, U.S.A. (now Millennium Pharmaceuticals, Inc., 75 Sidney Street,

Cambridge, MA 02139, U.S.A.), at the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110, U.S.A., under Accession No.[ ] PTA-990. The invention relates to murine hybridoma 7F3, to the antibody it produces, and to nucleic acids encoding the antibody.

Please replace the paragraph at page 33, line 28 through page 34, line 14 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

The invention also relates to a bispecific antibody, or functional fragment thereof (e.g., F(ab')<sub>2</sub>), which binds to a mammalian Bonzo and at least one other antigen (e.g., tumor antigen, viral antigen). In a particular embodiment, the bispecific antibody, or functional fragment thereof has the same or similar epitopic specificity as mAb 4A11, mAb 7A2, mAb 7F3 or mAb 9G2 and at least one other antibody. Bispecific antibodies can be secreted by triomas and hybrid [hybridoma]hybridomas. Generally, triomas are formed by fusion of a hybridoma and a lymphocyte (e.g., antibody secreting B cell) and hybrid hybridomas are formed by fusion of two hybridomas. Each of the fused cells (i.e., hybridomas, lymphocytes) produces a monospecific antibody. However, triomas and hybrid hybridomas can produce an antibody containing antigen binding sites which recognize different antigens. The supernatants of triomas and hybrid hybridomas can be assayed for bispecific antibody using a suitable assay (e.g., ELISA), and bispecific antibodies can be purified using conventional methods. (see, e.g., U.S. Patent No. 5,959,084 (Ring *et al.*) U.S. Patent No. 5,141,736 (Iwasa *et al.*), U.S. Patent Nos. 4,444,878, 5,292,668, 5,523,210 (all to Paulus *et al.*) and U.S. Patent No. 5,496,549 (Yamazaki *et al.*)).

Please replace the paragraph at page 34, line 15 through page 35, line 8 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

In another embodiment, the antibody or antigen-binding fragment thereof has specificity for a mammalian SExCkine, preferably a naturally occurring or endogenous human SExCkine. Such antibodies and antigen-binding fragments can be produced by a variety of suitable methods, such as



those described herein. In one embodiment, the anti-SExCkine antibody can be raised against an appropriate immunogen, such as an isolated soluble and/or recombinant SExCkine or portions thereof (including synthetic molecules, such as synthetic peptides). Antibodies can also be raised by immunizing a suitable animal (e.g., mouse) with cells which express the transmembrane form of SExCkine. In another embodiment, the antibody is an IgG or antigen-binding fragment of an IgG. In another embodiment, the antibody is a human antibody or an antigen-binding fragment thereof. In another embodiment, the antibody is a humanized antibody or an antigen-binding fragment thereof. In a preferred embodiment, the antibody or antigen-binding fragment can bind to a mammalian [sexcikine]SExCkine and inhibit (reduce or prevent) the binding of the chemokine to receptor (e.g., Bonzo), and thereby inhibit one or more functions mediated by receptor in response to SExCkine binding. For example, the anti-SExCkine antibody can inhibit SExCkine-induced chemotaxis of Bonzo<sup>+</sup> cells. Other functions which can be mediated by SExCkine binding to receptor (e.g., Bonzo) include, for example, signal transduction (e.g., GDP/GTP exchange by receptor associated G proteins, transient increase in the concentration of cytosolic free calcium  $[Ca^{2+}]_i$ ) and receptor-mediated processes and cellular responses (e.g., proliferation, migration, chemotaxis, secretion, degranulation, inflammatory mediator release (such as release of bioactive lipids such as leukotrienes (e.g., leukotriene C<sub>4</sub>)), respiratory burst).

Please replace the paragraph at page 38, lines 9 through 17 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

The first binding moiety can be, for example, [and]an antibody which binds mammalian Bonzo or antigen-binding fragment thereof (e.g., Fab, Fv, Fab', F(ab')<sub>2</sub>), a Bonzo ligand (e.g., mammalian SExCkine, mammalian platelet factor 4) or Bonzo-binding variant of a ligand. The second binding moiety can be, for example, an antibody or antigen-binding fragment thereof which binds to a molecule expressed on the target cell or antigen binding fragment thereof. Where the targeting molecule comprises a first binding moiety which is an anti-Bonzo antibody or antigen-binding fragment thereof, it is preferred that said anti-Bonzo antibody does not inhibit binding of ligand to Bonzo.

Please replace the paragraph at page 54, lines 15 through 17 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

In [a] another embodiment, the invention relates to a method of promoting Bonzo mediated homing of leukocytes in a subject, comprising administering an effective amount of a promoter (e.g., agonist) of Bonzo function.

Please replace the paragraph at page 57, lines 14 through 17 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

● immunosuppression, such as that in individuals with immunodeficiency syndromes such as AIDS, individuals undergoing radiation therapy, chemotherapy, or other therapy which causes immunosuppression; immunosuppression due to congenital deficiency in receptor function or other causes.

Please replace the paragraph at page 66, lines 17 through 27 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

Human multiple tissue northern blots I and II and a cancer cell line blot (Clontech) were used to [analyzed]analyze expression of the gene encoding the Bonzo ligand. cDNA probes were labeled with  $\alpha^{32}\text{P}$ -dCTP by priming with random hexamers. A 400 bp fragment representing most of the chemokine domain of SExCkine cDNA cloned in pCDEF3 (from the 5' EcoR1 site (within vector pCDEF3) to an EcoRV site of a cDNA encoding human SExCkine (SEQ ID NO: 3)) was used as the hybridization probe for all blots. Hybridization was performed at 68°C for 1 hour in ExpressHyb (Clontech) with denatured probe at a concentration of  $1 \times 10^6$  CPM/mL. Blots were then washed for 20 minutes in 2 X SSC/0.05% SDS at room temperature followed by high stringency washes at 50°C, 60°C, or 65°C in 0.1 X SSC/0.1% SDS for 20 minutes per wash and exposed to Kodak XAR film with an intensifying screen.

Please replace the paragraph at page 67, lines 3 through 7 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

Fusion proteins consisting of amino terminal regions of SExCkine fused to a C-terminal Histidine (His) were made in pEF-His or pEF1/V5-His A from Invitrogen (Carlsbad, CA) and fusion proteins consisting of N-terminal SExCkine regions fused to human alkaline [phosphates]phosphatase with a C-terminal His tag were made in the pDERF-SEAP vector (Yoshie, O., *et al.*, *J. Leukoc. Biol.*, 62(5):634-644 (1997)).

Please replace the paragraph at page 67, lines 8 through 16 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

The alkaline [phosphates]phosphatase fusion was produced by amplifying human SExCkine cDNA (SEQ ID NO:3) by PCR using a 5' synthetic oligonucleotide primer that contained a SalI site (5'cgcgctcgacagccgagatgggacgggacttg3', SEQ ID NO:12) and a 3' synthetic oligonucleotide primer that contained a XbaI site (5'ggtctagatgtcctggctgtgggacca3', SEQ ID NO:13). The 5' primer (SEQ ID NO:12) annealed to nucleotides 15-29 of SEQ ID NO:3 and encodes a protein beginning at the initiating Met (amino acid residue 1 of SEQ ID NO:4) and the 3' primer (SEQ ID NO:13) annealed to nucleotides 602-622 of SEQ ID NO:3. The PCR was run for 30 cycles (95°C (30 seconds), 55°C (30 seconds), 72°C (1 minute)).

Please replace the paragraph at page 67, line 25 through page 68, line 7 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

Additional constructs encoding fragments of the extracellular domain of [SexCkine]SExCkine were made by PCR using a 5' primer (SEQ ID NO:14) and synthetic primer KHLL 3' (5' ggt cta gaa agt aaa tgc ttc tgg tgg gc 3', SEQ ID NO:16) or synthetic primer LMS 3' (5'

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cct cta gag ctc atc aat tcc tga acc c 3', SEQ ID NO:17) or synthetic primer 155 3' (5' ggt cta gac tgg gag ggt ggg gcg ctg ag 3', SEQ ID NO:18). Primer KHLL 3' annealed to nucleotides 345-364 of SEQ ID NO:3, and the product of the amplification reaction encoded residues 1 to 117 of [SexCkine]SExCkine (SEQ ID NO:4). Primer LMS 3' annealed to nucleotides 280-300 of SEQ ID NO:3, and the product of the amplification reaction encoded residues 1 to 95 of [SexCkine]SExCkine (SEQ ID NO:4). Primer 155 3' annealed to nucleotides 457-477 of SEQ ID NO:3, and the product of the amplification reaction encoded residues 1 to 155 of [SexCkine]SExCkine (SEQ ID NO:4). Primers KHLL 3', LMS 3' and 155 3' each contained an XbaI restriction site.

Please replace the paragraph at page 68, line 19 through page 69, line 4 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

Thirty 10 cm plates (Beckton Dickinson) were seeded with  $1 \times 10^6$  293T cells in DMEM +10% FCS. The next day the 293T cells were transfected by adding 10  $\mu$ g SExCkine/SEAP DNA to 790  $\mu$ L opti-MEM (800  $\mu$ L total) and mixing it with a solution of 60  $\mu$ L LipofectAMINE™ 2000 in 740  $\mu$ L opti-MEM (800  $\mu$ L total). The mixture was incubated at room temperature for 30 min, an additional 6.4 mL of opti-MEM was added to the mixture, and the mixture was added to the plates containing 293T cells [with]. The plates were incubated at 37°C for 3 hours, then 8.0 mL DMEM+ 20% FCS was added. 24 hours later the transfection mixture was removed, the plates were washed with 1 X PBS, and 10 mL of serum free DMEM were added. The [Plates]plates were then incubated for 3 days. The media (culture supernatant) was removed and filtered (500 mL filter bottle) to remove cellular debris. The harvested media was assayed for chemotactic activity using Bonzo/L1.2 cells essentially as described above. In addition, dilution curves were generated using supernatant diluted in media in a range of undiluted to 1:16 to assess general activity.

Please replace the paragraph at page 70, line 19 through page 71, line 6 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

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A panel of antibodies which bind human Bonzo were produced by immunizing mice with transfected L1.2 cells that expressed high levels of Bonzo. The antibodies specifically bound to Bonzo expressed on the surface of Bonzo/L1.2 cells (Figures 8A-8D) but did not bind to transfected L1.2 cells which expressed CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, GPR5, V28, GPR9-6, Bob, LyGPR, AF, APJ or RDC (Figures 9A-9G). The antibodies, (e.g., mAbs 4A11, 7A2 and 7F3) inhibited the binding of SExCkine to Bonzo (Figure 10, Figure 31, Table 1). Staining studies revealed that Bonzo is expressed on small populations of CD4<sup>+</sup> and CD8<sup>+</sup> T cells as well as on CD16<sup>+</sup>/CD56<sup>+</sup> NK cells. However, no expression of Bonzo was observed on CD19<sup>+</sup>/CD20<sup>+</sup> B cells or on CD14<sup>+</sup> monocytes (Figures 11A-11H). Multi-color staining studies were performed to [analyzed]analyze the co-expression of Bonzo and other cell surface proteins (Figures 12A-12D, 13A-13J, 14A-14H, 15A-15C, 16A-16D). These studies revealed that Bonzo is expressed predominantly on CD45RO<sup>h</sup> memory lymphocytes. Furthermore, Bonzo expression was detected on both skin homing (CLA<sup>+</sup>) and gut homing ( $\alpha$ 4 $\beta$ 7<sup>+</sup> and  $\alpha$ E<sup>+</sup>) CD4<sup>+</sup> lymphocytes (Figures 12A-12D). Bonzo was co-expressed with CCR1, CCR2, CCR5, CCR6, CXCR1, CXCR2 or CXCR3 on lymphocytes (Figures 13A-13J).

Please replace the paragraph at page 72, lines 9 through 17 with the below paragraph marked up by way of bracketing and underlining to show the changes relative to the previous version of the paragraph.

This study demonstrates that recombinant SExCkine and recombinant proteins encoding parts of the amino terminal portion of SExCkine can be used in conjunction with Bonzo (e.g., a cell [expression]expressing Bonzo) in receptor binding assays and functional assays to screen for potential agonists and antagonists of Bonzo. Considering that Bonzo is highly expressed on all classes of chronically stimulated T cell subsets, antagonists of the receptor (e.g., mAbs 4A11, 7A2 and 7F3) can be administered to treat chronic inflammatory diseases. Furthermore, agonists of Bonzo (e.g., SExCkine) can be administered to recruit killer T cell subsets to, for example, solid tumors or sites of infection.

-X-

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

Claims 1-15, 17-20, 48-50, 52, 54-57, 59, 61-66, 68, 70-76, 78, 79, 81-83, 85-87, 95 and 96 have been cancelled and Claims 97-112 have been added.

24. (Amended) The antibody or antigen-binding fragment of Claim 21 wherein said antibody or antigen-binding fragment inhibits a cellular response to binding of ligand to said Bonzo in an *in vitro* assay with an  $IC_{50}$  of less than about [8] 7  $\mu\text{g/mL}$ .
27. (Amended) The antibody or antigen-binding fragment of Claim 21 wherein the binding of said antibody or said antigen-binding fragment to Bonzo can be inhibited by an antibody selected from the group consisting of mAb 4A11, the antibody produced by the hybridoma cell line deposited as ATCC Accession Number PTA-991, mAb 7A2, the antibody produced by the hybridoma cell line deposited as ATCC Accession Number PTA-992 and mAb 7F3, the antibody produced by the hybridoma cell line deposited as ATCC Accession Number PTA-990.
28. (Amended) An antibody produced by murine hybridoma 4A11, deposited as ATCC Accession Number PTA-991, or an antigen-binding fragment thereof.
29. (Amended) An antibody produced by murine hybridoma 7A2, deposited as ATCC Accession Number PTA-992, or an antigen-binding fragment thereof.
30. (Amended) An antibody produced by murine hybridoma 7F3, deposited as ATCC Accession Number PTA-990, or an antigen-binding fragment thereof.
31. (Amended) Murine hybridoma 4A11, deposited as ATCC Accession Number PTA-991.
32. (Amended) Murine hybridoma 7A2, deposited as ATCC Accession Number PTA-992.
33. (Amended) Murine hybridoma 7F3, deposited as ATCC Accession Number PTA-990.

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35. (Amended) The isolated cell of Claim 34 wherein said antibody or antigen-binding fragment inhibits a cellular response to binding of ligand to said Bonzo *in vitro* assay with an IC<sub>50</sub> of less than about [8] 7 µg/mL.
43. (Amended) The antibody or antigen-binding fragment of Claim 42 wherein said antibody or antigen-binding fragment thereof inhibits a cellular response in an *in vitro* assay with an IC<sub>50</sub> of less than about [8] 7 µg/mL.
44. (Amended) The antibody or antigen-binding fragment of Claim [41] 42 wherein said cellular response is chemotaxis.
45. (Amended) The antibody or antigen-binding fragment of Claim [21] 41 wherein said mammalian Bonzo is human Bonzo.
46. (Amended) The antibody or antigen-binding fragment of Claim [21] 41 wherein said ligand is SExCkine<sub>2</sub>.
84. (Amended) A test kit for use in detecting the presence of mammalian Bonzo or portion thereof in a biological sample comprising
  - a) an [agent] antibody or antigen-binding fragment thereof which binds to mammalian Bonzo [or portion of said receptor] and inhibits binding of a ligand to said mammalian Bonzo; and
  - b) one or more ancillary reagents suitable for detecting the presence of a complex between said [agent] antibody or antigen-binding fragment and said mammalian Bonzo [or a portion thereof, wherein said agent is not platelet factor-4].

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88. (Amended) The test kit of Claim [87] 84 wherein said antibody or antigen-binding fragment is selected from the group consisting of
- a) mAb 4A11, the antibody produced by the hybridoma cell line deposited as ATCC Accession Number PTA-991;
  - b) mAb 7A2, the antibody produced by the hybridoma cell line deposited as ATCC Accession Number PTA-992;
  - c) mAb 7F3, the antibody produced by the hybridoma cell line deposited as ATCC Accession Number PTA-990;
  - [b)] d) an antibody which can compete with mAb 4A11, mAb 7A2 or mAb 7F3 for binding to mammalian Bonzo; and
  - [c)] e) an antigen-binding [fragments] fragment of (a), [or] (b), (c) or (d) [which bind mammalian Bonzo or a portion thereof; and
  - d) combinations of the foregoing].

TO "SECRET"



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Michael J. Briskin, Kristine E. Murphy, Alyson M. Wilbanks and  
Lijun Wu

Divisional Application of:

Application No.: 09/449,437

Filed: November 24, 1999

For: NOVEL ANTIBODIES AND LIGANDS FOR "BONZO" CHEMOKINE RECEPTOR

Date: August 27, 2001

EXPRESS MAIL LABEL NO. EL 136 578819 U.S.

REMARKS

Box PATENT APPLICATION  
Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

The above-captioned application is a divisional of application number 09/449,437 filed on November 24, 1999 to which priority is claimed under 35 U.S.C. § 120.

On page 1, Attorney's Docket Number has been changed and page 1 has been numbered. The Sequence Listing is now presented as sheets 1/7 - 7/7 at the end of the Specification.

Amendments to the Drawings

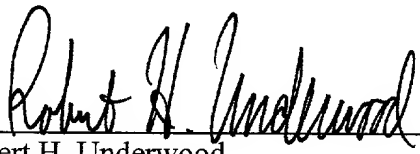
Formal Drawings are being filed with this application. These Formal Drawings are the same as the Formal Drawings that were filed in the parent application, U.S. Application No. 09/449,437. Formal Drawings 7, 10, 16, 18, 20-29, 31 and 32 have been amended to correct

informalities and typographical errors present in the Informal Drawings originally filed. Specifically, the spelling of "SExCkine" in Figs. 7, 18 and 27 has been corrected; the spelling of "µg/ml" in Figs. 10, 21, 31 and 32 has been corrected; the spelling of "IgG2a" in Fig. 16C has been corrected; the spelling of "MIP-3 alpha", "RANTES" and "Day 7 IL2" in Fig. 18 has been corrected; the spelling of "% Bonzo-positive Cells" in Figs. 20 and 22 has been corrected; the spelling of "T<sub>H</sub>1", "T<sub>H</sub>2" and "T<sub>R</sub>1" in Figs. 23, 24, 25, 26 and/or 27 has been corrected; the spelling of "RANTES" in Fig. 27 has been corrected; the spelling of "Background" in Figs. 28 and 29 has been corrected; and the spelling of "pCDEF3" in Fig. 29 has been corrected.

No new matter has been introduced by these changes.

Respectfully submitted,

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094003.030450